Review article

BSE: a decade on—part I

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Bovine spongiform encephalopathy (BSE), popularly known as "mad cow disease", was discovered in 1986 and has accounted for the deaths of over 165 000 cattle in the UK (by the end of January, 1997) with about 34 000 (mainly dairy) herds involved. The syndrome in the cow includes changes in posture and temperament, apprehension, and loss of coordination. There are many parallels with scrapie in sheep, with similar neuropathological changes in the hindbrain that give it a spongiform appearance under the microscope. The facts have been broadly reviewed in *The Lancet* in 1990 and 1993, and in much more detail elsewhere. In a two-part article, the first of which appears here, we now summarise recent developments.

The UK outbreak

The first cases of bovine spongiform encephalopathy (BSE) seen in 1985–86 were probably infected as calves in the winter of 1981–82 (see refs 1–4 for reviews). Figure 1 shows how the epidemic developed. The modal age of occurrence is 5 years (range 20 months to 18 years), and at the time of initial assessment[1] the incubation period was estimated to be 2·5 to at least 8 years. Most cases (figure 2) were infected as calves. Adult dairy cattle were predominantly affected, because as calves they had received the meat-and-bone meal that, we believe, carried the agent in cereal-based concentrate rations and because they had generally lived long enough to develop the disease. Bull calves derived from dairy cows are nearly always castrated and slaughtered for beef at around 2 years of age, so, even if they were infected via meat-and-bone meal, disease would not usually have had time to be expressed. The disease typically occurred in only a few animals in any one herd (mean size 80 animals). Increased herd size increased the risk (see below) and some birth cohorts in large herds were very severely affected.

Over the decade, 35% of farms experiencing BSE have had only one case, and 69% have had four cases or fewer. The low average within-herd incidence (<3% in any 6-month period since the epidemic began) is attributed to a low average exposure of the cows to "packets" of infectivity that were generally widely spaced in different batches of feed.[6] It has been calculated that the average exposure in affected herds may have been as low as 14 oral LD50 per tonne of concentrate feed[6] (LD50 is the dose needed to kill 50% of a test group of animals). Since the within-herd incidence of infection would have been higher than the within-herd incidence of expressed disease, we should be cautious in accepting this figure.

A small-herd owner purchasing small amounts of concentrate would have been less likely to have bought infected feed, whereas owners of large herds would have bought large amounts carrying only a sufficient number of infectivity packets to result in the generally low within-herd incidence. However, the incidence within a cohort could be much higher than the general within-herd incidence and could occasionally be 100%. Such an event could be attributed to purchase of a batch of concentrate feed that contained meat-and-bone meal prepared from raw material with a disproportionately high amount of BSE or scrapie-infected tissue that had been inadequately inactivated by rendering. Large purchases of such a batch by a large-herd owner who fed it to calves destined for breeding in his herd would then have been more likely to have had a very high incidence of disease in the cohort, especially if the meal was not well mixed.

The early pattern of occurrence across the UK was that of an extended common-source epidemic, with the

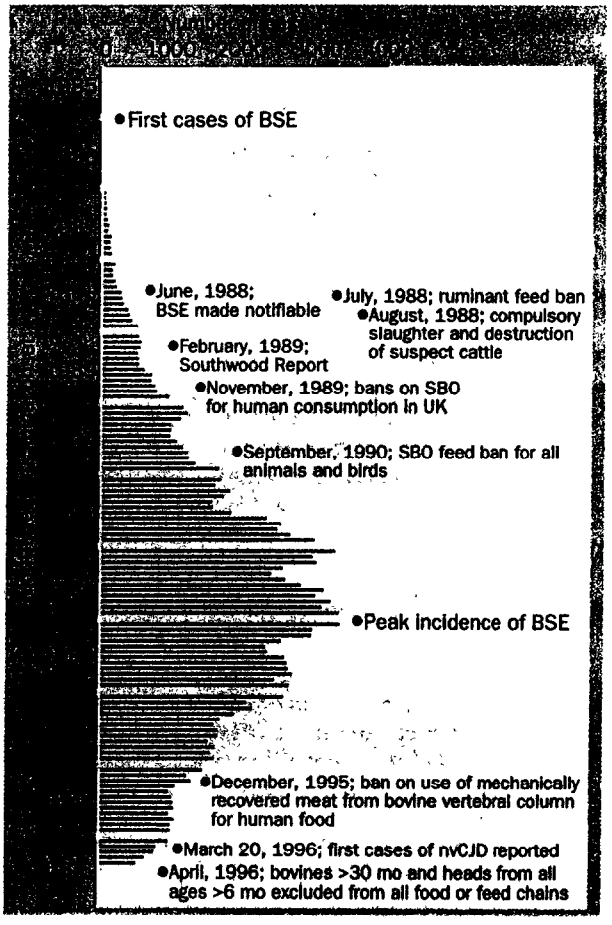

Figure 1: **Confirmed cases of BSE by month and year of clinical onset in Great Britain, 1986 to May, 1996**
Courtesy of MAFF.

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incidence highest in the south and east of England, lowest in Scotland, and initially absent from Ireland. The vehicle of infection was identified as ruminant-derived protein in meat-and-bone meal supplied from rendering plants that processed rejected animal carcasses and animal waste from abattoirs and butchers. The peak incidence was in late 1992 and early 1993; thereafter, there has been a progressive reduction in the annual incidence. The fall in incidence by age-class began first in 1991 in the 2-year-old age class and was progressive (3-year-old age class in 1992, 4-year-olds in 1993, and so on) and this was sustained.

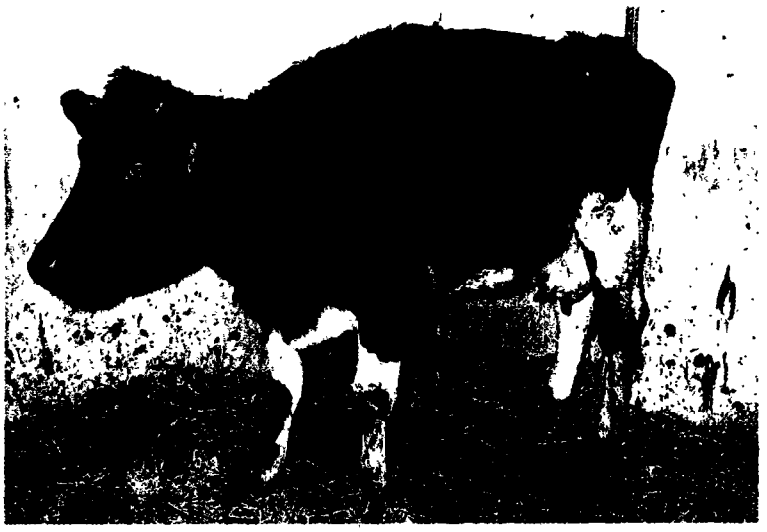

Figure 2: **A Holstein-Friesian cow with BSE**
Shows apprehension with typical low head carriage, arched back and wide-based stance. Courtesy of K F D Brown.

BSE elsewhere

Fewer than 50 cases of BSE have been reported in cattle bred in the UK and imported into Canada, Denmark, the Falkland Islands, Germany, Ireland, Italy, the Sultanate of Oman, and Portugal. That there are not more is surprising, since more than 50 000 pure-bred breeding cattle were exported from the UK in the critical period after 1985. A few cases have been diagnosed in native cattle in France, Ireland, Portugal, and Switzerland, mainly assumed (but not all unequivocally) to be due to consumption of infected meat-and-bone meal of possible UK origin in concentrate rations. By December, 1996, Switzerland had reported 234 cases of BSE—the greatest number for any mainland European country to date. Again, it is surprising that there are not more cases in the other countries in view of the large quantities (>71 kilotonnes) of meat-and-bone meal exported from the UK up to 1990 for use in pig and poultry feed, and in view of the risk of cross-contamination of ruminant diets with these commodities or with meat-and-bone meal itself in mills or on farms where mixed species feed was prepared. Until October, 1990, exported meat-and-bone meal could have included protein from specified bovine offals (SBO). In the UK, such SBO-containing meat-and-bone meal was fed to pigs and poultry until September, 1990, and there is no reason to doubt that this was happening in other countries even after 1990.

The range of concern

The catastrophic outbreak is of major economic importance to British farmers, cattle breeders, meat and meat-product dealers, butchers, and pharmaceutical interests related directly or indirectly to products of bovine origin. Anxieties have extended to so-called catgut sutures made from bovine intestine, to fetal calf serum and other bovine products used for the growth or stabilisation of bacteria and viruses used in vaccines, to bovine materials used in implants or injections, and to the use of gelatin in the pharmaceutical and food industries. Risk assessments have been conducted and controls instituted to protect public and animal health from these potential or actual hazards, but there has been much criticism of their apparent inadequacy. In fact, bovine materials used in the preparation or final product of pharmaceuticals, medicines, and devices manufactured in the UK have been sourced from outside the UK since BSE was recognised so that any theoretical hazard was avoided. There was much speculation that BSE may be transmissible to man by ingestion of beef. This is very unlikely because, despite the limitations of the mouse bioassay used to assess such transmissibility, infectivity has never been found in muscle in any naturally occurring transmissible spongiform encephalopathy, including BSE. Infected central nervous tissue (brain, spinal cord, or eye) could theoretically be a source, however. Many authorities believed it unlikely that BSE would be transmissible to man in food.[7-9] The opposing views of Lacey and Dealler and others[10-12] were not respected by the establishment (including us). Dealler and Kent calculated, however, that significant dietary exposure of the UK population to the BSE agent *has* occurred in the past;[12] some think that, without hard data, the term "significant dietary exposure" may be incorrect. No definite link has yet been made, but it is now feared that a hitherto unknown pattern of Creutzfeldt-Jakob-like disease (new variant CJD, nvCJD) recently recognised in ten patients aged less than 42 years in the UK[13] and one from France[14] could possibly be related to exposure of these patients to the BSE agent during the years before control measures relating to bovine products in the food chain were effective. Diringer[15] postulated that there may be a link between scrapie in sheep, BSE in cattle, and CJD in man. These and other diseases with similar pathology (eg, kuru) are collectively known as transmissible spongiform encephalopathies (TSE) (see panels 1 and 2). The transmissibility and lengthy incubation periods of CJD were not appreciated until the late 1960s and, because of incomplete ascertainment, precise data on case numbers are probably only available in a few countries such as the UK and Austria in the present decade.

Early official action

The British government made BSE a notifiable disease in June, 1988, introducing in July, 1988, a temporary ban (until December, 1988, subsequently extended for 1 year) on the supply and use of ruminant-derived protein in ruminant feed. It set up a Working Party on BSE under the chairmanship of Sir Richard Southwood and, when their report, published in February, 1989,[22] recommended that the feed ban be continued indefinitely, this was done. In August, 1988, the compulsory slaughter and disposal of carcasses of all cattle suspected of having BSE was

Panel 1: **Chronology of naturally occurring TSEs of man and animals**

Host	Disease	First reported
Man	Kuru	1957
	CJD and variants	1920/1928–92
	nvCJD	1995-96
Sheep	Scrapie	1730
Goats		1872
Mouflon*		1992
Mule deer	Chronic wasting disease	1967
Elk		
Ranch-bred mink	Transmissible mink encephalopathy	1965
Cattle	BSE	1986
Exotic ungulates (in zoos)	SE in captive nyala, gemsbok,* Arabian oryx,* scimitar-horned oryx,* greater kudu, eland,* ankole*	1986–95
Domestic cats	Feline SE	1990
Big cats (*Felidae*) (in zoos)*	SE affecting pumas, cheetahs, ocelots, tiger	1992–96
Macaque	SE	1996

*Transmissibility not formally established in these species.
SE=spongiform encephalopathy.

ordered, with 50% compensation paid (subsequently increased to 100% in February, 1990). In December, 1988, all milk from suspect animals was ordered to be destroyed, but, for welfare reasons, an exception was made for feeding a cow's own calf. In fact, neither milk nor mammary gland (udder) has ever shown detectable infectivity in naturally occurring TSEs including BSE. By February, 1989, all of the recommendations of the Southwood Report had been acted upon (see MAFF Report May, 1996[23]).

The BSE agent

The BSE agent in cows causes characteristic neuropathological changes in the hindbrain (figure 3); it is transmissible by parenteral injection of infected brain homogenate into cattle, sheep, goats, pigs, mink, marmosets, and mice, but not hamsters or chickens. The agent is experimentally transmissible by the oral route, in some cases with very high-challenge doses only, to calves, sheep, goats, mink, and mice, but not to pigs at least up to 6·5 years post-challenge (GAH Wells, Central Veterinary Laboratory, Addlestone, Surrey, UK). Transmission orally to sheep and goats was possible with 0·5 g of infected bovine brain; and in 1995 it was discovered that as little as 1 g of brain was effective in cows. Oral challenges with less than 1g of brain have not yet been tested in cows. Action was taken in August, 1995, to prevent removal of brains and eyes from bovine skulls. In April, 1996, following advice from the Spongiform Encephalopathy Advisory Committee (SEAC), the entire head, excluding the tongue, was compulsorily removed from all food and feed chains. The agent has not been visualised and does not have the characteristics of a bacterium or a conventional virus. In these respects, and in its resistance to chemicals, heat, and radiation (in tests with preparations of infected brain tissue), it behaves like the scrapie agent.[24]

The scrapie agent

The scrapie agent has been passed through cell cultures in some experimental studies, but it has not been cultured efficiently in any system. The agent is experimentally transmissible from sheep to sheep and goats, and to mice, hamsters, rats and a range of other species including cattle.[25–28] The experimentally transmitted cattle disease produced by parenteral injection of US scrapie-infected material is, however, clinically and pathologically different from naturally occurring UK BSE; in this context, there is no convincing proof to link natural scrapie in sheep with the currently recognised syndrome of BSE in cattle. In negatively stained electronmicroscope preparations of detergent extracts of affected brain treated with proteinase K, scrapie-associated fibrils (SAF) occur[29,30] (figure 4). These are composed of an abnormal form of host glycoprotein called prion protein (PrP). The normal form is called PrP^{C} (cellular) and the abnormal form in scrapie-like diseases is designated PrP^{Sc} and is relatively resistant to proteinase K (hence, the use of PrPres as a general term now). Prusiner refers to aggregates of prions as prion rods[31] (see below), and many regard these as equivalent to SAF. When SAF are purified, there is some co-purification of infectivity (ie, increase in titre), but some workers do not equate SAF directly with the transmissible agent. There are differing views on whether SAF occur naturally in vivo or are artifacts produced by the laboratory processing of material for electronmicroscopy.

The nature of the TSE agents

The infectivity of affected scrapie brain homogenate passes through small-pore filters (eg, 50 nm). In tests with 10% homogenates of BSE-infected bovine brain or scrapie-infected rodent brain, the agent is not destroyed by boiling in water, and it is not inactivated by standard exposures in an autoclave to wet heat at 121°C for 15 min. Exposure to 134–138°C for 18 min in a porous-load autoclave is currently recommended but may not be adequate in all circumstances, though no problems have been reported in practice (ref 32 and D M Taylor, Institute for Animal Health, Edinburgh, UK). The scrapie agent withstands alcohol and strong disinfectants such as formaldehyde and glutaraldehyde. Formaldehyde may even increase its heat-stability. It may be inactivated by exposure for 1 hour to sodium hypochlorite providing 2% available chlorine; although 1N or 2N NaOH is a useful decontaminant, neither concentration is inactivating after 1 hour.[32–35] The scrapie agent has been the model for many of these inactivation studies, but the agents of BSE and

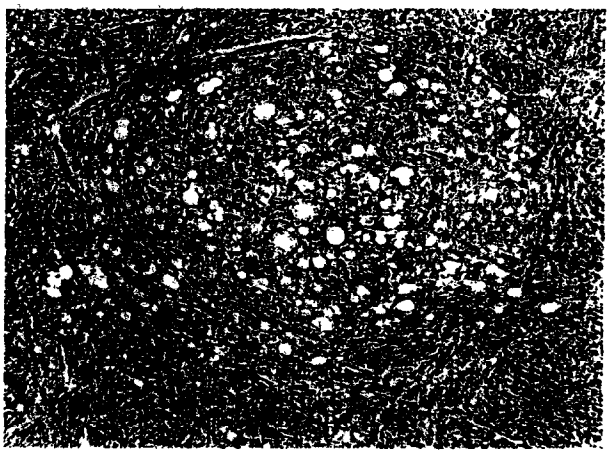

Figure 3: **Neuronal vacuolation and spongiform change in the brain of a cow with BSE**
Haematoxylin eosin ×128 reduced by one-third. Courtesy of G A H Wells and S A C Hawkins.

Panel 2: TSEs of man and animals

Man

Kuru occurred in the South Fore tribe of Papua New Guinea and predominantly affected women and young children. The disease was linked to endocannibalism as a mourning ritual and to smearing of the remains of the deceased on the skin of the mourners.[illegible] The women and young children were selectively exposed to brain, internal organs, and intestines in the ceremony. There is no evidence of secondary cases or of prenatal, perinatal, or postnatal transmission of kuru from mother to child, thus supporting the view that milk does not transmit infectivity; a direct route of infection seems to be necessary. The incubation period ranges from [illegible] years to more than 30 years.[illegible]

CJD has an annual worldwide incidence of 1 in 1–2 million persons, with sporadic cases accounting for about 85% of the total. Some workers claim that the sporadic form is caused by spontaneous somatic mutation in the prion protein (*PrP*) gene with the production of abnormal PrP. An alternative theory is that it may result from a switch to neurovirulence in a silent, harmless (ie, latently carried) ubiquitous strain of the CJD agent,[illegible] or that the normally harmless form is not harmless in a small number of genetically susceptible individuals. Inherited, familial, genetically predisposed cases contribute about 12–15% of cases with germ-line mutations of varying penetrance. Geographical clusters of this form due to interbreeding are well documented. Iatrogenic CJD has been associated with injections or implantations of materials derived from unknowingly CJD-infected cadavers or from neurosurgical electrodes or instruments contaminated from a previous case and inadequately sterilised. The resistance of the CJD agent is similar to that of the scrapie agent; special precautions must be taken[illegible] that are now well implemented, so new exposures of this sort should not occur.

Animals

Transmissible mink encephalopathy, a rare disease affecting ranch-bred mink, was first recognised in 1947 in Wisconsin, USA.[19,20]

Chronic wasting disease of captive and wild mule deer and elk is also a rare disease and has only been observed in North America.[21]

BSE-like disease has occurred in 18 captive wild ruminants of seven different species born and reared in the UK up to Jan 1, 1997 (D Matthews, MAFF, Tolworth, Surrey, UK), presumably as a result of ingestion of the same ruminant-derived protein in feed concentrates that caused BSE in our cattle.

Feline spongiform encephalopathy (FSE) was discovered in domestic cats in the UK in 1990. Up to January, 1997, 76 cases of FSE have been confirmed in cats in the British Isles, one in a cat in Norway, and one in Lichtenstein. The source is probably feed, but the precise origin has not been identified. FSE also occurred in captive large cats (four cheetahs, three pumas, two ocelots, and a tiger up to Jan 1, 1997) born in UK zoos and presumably unknowingly fed BSE-infected raw central nervous tissue from heads or necks of fallen cattle before September, 1990, when the law was changed to prevent such an occurrence (D Matthews, MAFF).

scrapie behave similarly. Preparations of infected brain (brain homogenates or macerates) have been used in such tests. It should be noted that brain, with its high content of protective lipid, would be regarded as a relatively protective medium in conventional microbiological resistance tests, and macerates are more difficult to decontaminate than whole brain or homogenates.

The infections all have typically long incubation periods. The terms viroid, virino, and prion have been successively coined to take account of evolving views on unconventional transmissible agents and their unusual biological behaviour (see Kimberlin[17] and Schreuder[36]). Many but by no means all workers regard the TSE agents as prions, which are proteinaceous infectious particles apparently devoid of nucleic acid,[31] and thus the TSEs are also referred to as prion diseases. Prion theory is still controversial. Different isolates of scrapie agent can be characterised (or "strain typed") in experimental mice according to the nature and distribution of the neuropathology and the incubation periods of the disease that they produce in various inbred strains (*Sinc* s7 and p7 genotypes and an F_1 cross). In the sheep and the mouse, the incubation period of scrapie agent is influenced by host genes (*Sip* and *Sinc*, respectively). However, scrapie strains also have apparently "genetic" characteristics of their own in relation to their pathogenicity. More than 20 different strains of scrapie can be characterised by current tests in mice,[37] but a nucleic acid genome that could explain such a feature has not been identified.[36,38,39] The BSE isolates obtained so far from cows in different parts of the UK and even in Switzerland all behave similarly when studied in mice.[40,41] To this extent, BSE stands apart from scrapie; it seems that a stable bovine-adapted strain of BSE dominates in the bovine disease in the UK.

The source of BSE

Meat-and-bone meal is produced by rendering discarded animal fat, bones, offal, whole carcasses, and other "mixed material" from bovine, ovine, porcine, poultry, and other sources. This meal had been incorporated into cattle feed since the 1940s and until the feeding of ruminant-derived protein to all ruminant species was banned on July 18, 1988. At that time there were 46 rendering plants in the UK.[42] The raw materials were broken down and processed in several different systems at temperatures ranging from less than 100°C to 150°C for varying periods. The molten fats (tallow) were then separated from the protein-rich solids (greaves) from which the protein supplement meat-and-bone meal was produced by grinding. The crude greaves at this stage would contain up to 14% fat, but further processing of greaves had traditionally included a hydrocarbon extraction step to increase the yield of tallow by prolonged further heating in the presence of

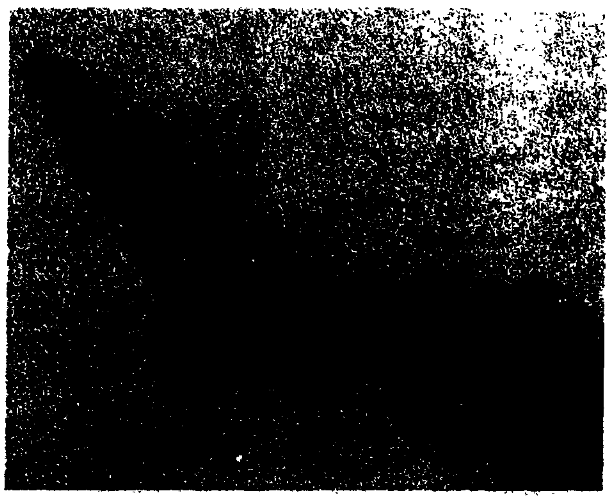

Figure 4: **BSE fibrils analogous to scrapie-associated fibrils (SAF) in an electronmicroscopy preparation of infected bovine cervical spinal cord**
N-lauroylsarcosine detergent extraction and proteinase K digestion, followed by negative contrast transmission electronmicroscopy ×125 000 (reduced by 40%). Courtesy of M J Stack.

hydrocarbon solvent (at about 70°C for 8 hours). The extracted greaves contained less than 1% fat. Residual solvent was removed from the extracted greaves by treatment with pressurised steam ("live steam stripping"). The solvent extraction and solvent recovery steps were omitted in the late 1970s and early 1980s in most plants because the market for tallow declined, and also for safety reasons. Accordingly, any infective agents present in the ingredients after that change would not have been subjected to the hot solvent extraction or the solvent recovery steps and would have been likely to have been more protected in greaves with a relatively high fat content.[35] The hasty omission of the solvent step by English producers recorded during 1981–82 covers the period when it is thought that the BSE challenges were delivered to the cows that first went down with BSE in 1985–86.[42]

It is likely that the present outbreak of BSE was derived from scrapie-infected sheep as a result of an increase in the dose presented when the rendering process was altered. The lack of formal proof of this hypothesis by experimental challenge of BSE-free cattle with British strains of scrapie agent may seem surprising. However, such an experiment—whatever its results—would not affect animal or public-health controls. It would be difficult to design a likely experiment. What strain of scrapie would we use? What genotype of cattle would we select for the challenge? For how many years would the experiment run? It is accordingly understandable that the priority for this study was deemed to be lower than that for many others, though it may now merit reconsideration. BSE was undetected until November, 1986, because the incidence was too low to attract attention.[6] Nevertheless, the recycling of infection from cattle boosted the epidemic from mid-1989 onwards, when material from undiagnosed cases was rendered and included in ruminant feed in the period from about 1984 to July, 1988. Irrespective of whether the agent came from sheep or directly from cows, the infection would have been passaged and boosted through cattle via supplemented feed until the ban on ruminant-derived protein was imposed and became fully effective. If the BSE agent evolved in the cow from the ovine scrapie agent, the continuing selective pressure of passage in the new host species would ensure the emergence of a bovine-adapted strain with a relatively short incubation period, and this would dominate in our cattle. Kimberlin has shown how this can happen with experimental models of scrapie in mice and how selection of a mutant strain of scrapie can occur when the species barrier is crossed.[43]

If the BSE agent was primarily bovine in origin, might it nevertheless have been recycled in sheep as well as cattle in the past decade? At present, there is no evidence that BSE has transmitted naturally back to sheep, but some sheep were fed meat-and-bone meal until this practice was banned in 1988, and BSE has been transmitted experimentally to sheep by intracerebral inoculation and by oral dosing with brain tissue from cows with BSE.[44,45] If feed exposure to the BSE agent occurred and was perpetuated in sheep either by accidental cross-contamination of sheep diets with infected meat-and-bone meal (as cattle diets were) or by maternal and horizontal transmission, as in scrapie, it is theoretically possible that BSE could have become endemic in a flock.

Some factors reduce this possibility: UK sheep were generally fed much less concentrate feed than were dairy cattle, and often none at all. Concentrate feeding is largely restricted to specialised groups of sheep and fed for the first time usually when adult. An exception would be early-born lambs reared for the Easter trade, but these would present a minimum risk; first, the agent would have little time to replicate sufficiently to produce an infectious dose; and, second, these sheep would be dead-end hosts. In fact, meat-and-bone meal was not invariably a constituent because sheep are more fastidious feeders. The ruminant feed ban of 1988 would have reduced sheep exposures from feed, as it did for cattle, and transmission of BSE in sheep might be further restricted by the *PrP* genotype or perhaps by other genes influencing the susceptibility and resistance of sheep.

If there are any risks from the hazard of BSE in sheep, they are currently unquantifiable. Any foodborne risk would be greatest from those tissues containing the agent in the highest titre, namely the brain and spinal cord of older sheep. A lower infectivity titre would be expected in earlier stages of incubation in the spleen, the only non-cerebral tissue so far tested for BSE infectivity in experimentally challenged sheep,[45] and perhaps other lymphoreticular tissues, which are not normally included in the UK diet. Over recent centuries there is no evidence linking sheep scrapie with human disease; accordingly, there are no findings on record to suggest that sheep prions (ovine PrP^{Sc}) have ever caused any human TSE. We take account of Diringer's point[15] that the BSE agent may have increased (cross-species) pathogenic potential, but scrapie/TSE agents have been transmitted to more than 30 species. More have been experimentally challenged with and succumbed to scrapie than with and to BSE. Natural disease has occurred in more species with BSE than with scrapie, but the BSE "escapes" are all attributable to infection from feed as the common source. This is not an example of species-jumping in the recognised sense. We have had no recent epidemic of scrapie in sheep to parallel that of BSE in cows, though we do not have accurate incidence data for scrapie.

Part 2 will appear in the March 8, 1997, issue of The Lancet.

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